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From concretes to bioreceptive concretes, influence of concrete properties on the biological colonization of marine artificial structures

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Abstract. A main cause of biodiversity loss is artificialization of the marine environment (IPBES 2019). With 39,400 km² of coastal and marine areas already encroached upon by human infrastructure and an increasing demand on space due to the growing global population – projected to reach 9 billion by 2050 – it is clear that humanity needs to find ways to prevent its activities from endangering biodiversity. To this end, since the 1990s ecologists have been trying to develop a win-win approach that unites ecological engineering with civil engineering. Today, civil engineers have a responsibility to incorporate eco-design processes in all construction projects underway to ensure that the latter benefit both humans and nature. Then, the new challenge of the 21st century is to develop eco-designed concretes that, in addition to their usual properties, provide improved bioreceptivity in order to enhance marine biodiversity without affecting the structure durability. The aim of this study is to master, clarify and classify the intrinsic parameters that influence the bioreceptivity (biocolonization) of cementitious materials in the marine environment. By using biofilm-culture-method (biofilm quantification), this study shows that the use of rough surface or slag cement CEM III and the surface treatment with green formwork oil enhance the biocolonization of cementitious materials in the marine environment whereas the application of curing agent (hydrophobic surface coating) has the opposite effect. Among the influent parameters, surface roughness proved to be the factor that promotes biocolonization most effectively.

Key words: Eco-design; cementitious materials; marine environment; Intrinsic parameters; Biocolonization, Bioreceptivity

1. Introduction

In the marine environment, microbial cells are often found in complex, surface-attached communities, known as biofilms. By definition, biofilms are assemblages of single or multiple microbial populations irreversibly attached to a substrate or an interface and included in a coating of extracellular polymeric substances (EPS) [1]. Biofilms arbor considerable microbial diversity. Bacteria, archaea, algae, fungi, protozoa and viruses all form important components of the biofilm matrix and dominate biogeochemical and bioremediation processes in seawater [2,3]. Then, biofilms contribute to the biodiversity and ecosystem processes of aquatic ecosystems.

Moreover, any surface in the marine environment will be colonized with micro- and macro-organisms leading to biofilm formation [4]. This biocolonization process develops via four distinct phases; adsorption of dissolved organic molecules, attachment of bacterial cells, attachment of unicellular eukaryotes and attachment of larvae and spores [5]. Bacterial attachment is a highly controlled and regulated process whereby attached cells produce EPS to form structured and complex matrixes [6]. Then, these pioneer microorganisms facilitate the establishment of the next arrivals, including heterotrophic and photosynthetic microorganisms such as cyanobacteria, fungi, diatoms, barnacles, algae and protozoa [7].

However, in alignment with the global attention to environmental protection and biodiversity restoration in the marine environment [8], the number of studies about the biocolonization of submerged materials and their effects on marine ecology and biodiversity has increased in the last decade. Indeed, in the marine environment, urban, coastal and offshore structures provide an important protective function, but can also have unintended ecological consequences, such as the loss or modification of habitat and the alteration of hydrological and ecological flows [9,10]. The new challenge of the 21st century for managers is to eco-design marine infrastructure in a way that minimizes its ecological impact and increases its bioreceptivity (the ability to be colonized by living organisms) in order to enhance marine biodiversity without losing the structure durability [11]. Knowing that concrete is one of the materials most widely used for the construction of marine infrastructure such as ports and coastal defences [12], master and clarify the intrinsic parameters that influence the bioreceptivity of cementitious materials in the marine environment are crucial and constitute a fundamental step towards more green and eco-designed marine artificial structures.

However, the durability of a concrete structure in the marine environment is influenced by the chemical, physical and biological actions [13]. Influences of a physical and chemical nature are generally well studied, and are subject to standards and recommendations [14]. Several scientific publications state that actions of a chemical nature such as chloride and magnesium sulfate attack are the main cause of concrete structure deterioration in the marine environment [13,15]. However, the actions of a biological nature (e.g., colonization of concrete by marine organisms) have been less studied, and less is known about them [14]. The effect of these actions on the durability of concrete in the marine environment remains unclear, but most scientists agree that marine organisms adhered to the concrete surface have a protective effect (bioprotection) against chemical attack in seawater [16–18]; they form a physical barrier that reduces surface permeability. The decrease in surface permeability leads to less-efficient diffusion of aggressive ions (Cl^-, Mg^{2+} and OH^-), which can increase the durability of a concrete structure in the marine environment [19,20].

Generally speaking, the intrinsic parameters that influence the biocolonization (biofilm formation) of cementitious materials are the nature and the physicochemical properties of the surface: chemical composition, roughness, porosity, hydrophobicity and pH [21–27]. However, these factors are less well known in the marine environment. We showed in a previous study that the bacterial colonization of cementitious materials in seawater is influenced by the pH and the type of cement [28,29].

In order to help engineers in their new challenge of designing marine infrastructures using eco-designed concrete, the main objective of this present study is to determine the effect of some intrinsic parameters on the biocolonization of cementitious materials in the marine environment. These parameters are the the use of curing compound or green formwork oil, the surface roughness and the type of cement.

2. Materials and Methods

2.1. Preparation of Mortar Specimens

Ordinary Portland Cement (OPC) CEM I cement 52.5 N CE CP2 NF "SB" (provided by Ciments Calcia) and blastfurnace slag cement CEM III (composed of 60% ground granulated blast-furnace slag NF EN 15167-1, provided by ECOCEM (CAS no.: 65996-69-2) were used in this study to produce six types of mortar specimens (Table 1). The mortar had a water/cement ratio (w/c) of 0.5 and was composed of 450 g cement and 1350 g sand (sand 0/4 EN 196-1).

Table 1. Types and compositions of mortar specimens investigated in this study.

Mortar Specimen		**Surface Type**	**Cement**	**CEM I (g)**	**CEM III (g)**	**Water (g)**	**Sand (g)**	**Curing Agent**	**Formwork Oil**
Control mortar	Ref	Smooth (SS)	CEM I	450	0	225	1350	-	-
Cured mortar	CM		CEM I	450	0	225	1350	+	-
Oiled mortar	OM1		CEM I	450	0	225	1350	-	+
	OM3		CEM III	180	270	225	1350	-	+
Biomimetic mortar	BM1	Rough (RS)	CEM I	450	0	225	1350	-	-
	BM3		CEM III	180	270	225	1350	-	-

After mixing, the mortars were cast in cylindrical molds measuring 2.7 cm in diameter and 2.9 cm high. After 24 h of hardening, the mortar specimens were removed from the molds and placed in a laboratory room at 20 °C for 30 days. Formwork oil (vegetable oil, BIODEM SI 3) was spread on the molds, using absorbent paper to avoid any surplus, before the mortar was poured. Curing compound (SikaCem®Cure) was added to the top of the specimens in accordance with the supplier's instructions 1 h after stripping. The rough mortars were prepared with a rough silicone skin; no release agent was needed. The cylindrical molds filled with mortar were poured on the silicone skin and held in place with a weight.

2.2. Immersion Conditions

An immersion test in seawater was carried out using flat-bottomed basins (polyester, 6 m long, 0.6 m high and 2 m wide) located at the IFREMER station (biology of exploited marine organism's research unit in Palavas, France). The basins featured a seawater inlet and outlet allowing for an open seawater circuit. To ensure that the experiment would be completed smoothly and avoid contamination of any type, the basins were cleaned before the cementitious material specimens were placed in them.

2.3. Quantification of bacterial colonization

Quantification of the bacterial biofilm adhered to the surface of the mortar specimens was performed using the protocol described by Hayek *et al.*, (culture-based methods) [28]. This quantification was carried out after 0, 1, 2, 6, 8, 15, 24, 26 and 28 days of immersion in seawater. After each incubation period, three specimens of each mortar type were placed in three sterile tubes containing 10 mL of sterile seawater. Bacteria adhering to the mortar surface were detached by immersing the tubes in an ultrasonic bath (Bandelin SONOREX™) for 10 min at 20 °C. The solution obtained was diluted using sterile seawater and 100 µl of diluted solution were spread on plates containing marine agar (MA, Dutscher, 490614). The plates were then incubated at 20 °C, and a colony count was performed at least 72 h after incubation. The results are expressed as colony-forming units per cm^3 of mortar (CFU/cm3).

2.4. Hydrophobicity Evaluation

The hydrophobicity of the mortar surface during bacterial colonization was evaluated using a drop-shape scanner (KRÜSS, DSA 30), which measures the angles and diameters of contact between a drop of water and a surface [30]. After each period of incubation in seawater, 3 mortar specimens were drained for 2 h. Then, a drop (3 µL) of water was placed on mortar surface. Absorption of the drop was monitored using a camera (8 images per second). The contact angle between the drop and the material was measured using the Advance machine software, and a minimum of three tests were carried out at different points.

2.5. Statistical Analyses

In order to evaluate the significance of the various biocolonization obtained, statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) and one-way ANOVA tests. Statistical significance was accepted by p-value <0.05 obtained using Bonferroni or Tukey's multiple comparison post hoc tests.

3. Results and discussion

The biological colonization of cementitious materials is affected according to the literature by many factors, including the environmental conditions, the bacterial properties and the physical/chemical characteristics of the material surface [24,31]. To avoid the effect of the environmental conditions and bacterial parameters on the biocolonization results, the mortar specimens were incubated at the same time under the same environmental conditions in the presence of the same marine bacteria. Therefore, only the physicochemical properties of the mortar surfaces could generate a different rate of bacterial colonization.

3.1. Effect of the curing compound and the green formwork oil on the biocolonization of mortar specimens

Generally, the curing compound and the green formwork oil are used during manufacturing to improve the durability of concrete; the curing agent is a liquid applied to the concrete or mortar surface to protect the material from water evaporation and give it greater aesthetic and mechanical durability, preventing early-age surface cracking [32]. Formwork oil is a mold-release agent that is applied to a wall of a mold to ensure easy separation of the hardened concrete from the mold by reducing the adhesion forces at the concrete/mold interface [33]. This oil forms a well-adsorbed and stable "lubricant monolayer" on the surface of cementitious materials, leading to improved release performance [34].

The effect of this tow products (curing compound and green formwork oil) is poorly understood to date. Figure 1 shows that the curing compound inhibits the bacterial colonization of mortar submerged in seawater whereas green formwork oil has the opposite effect.

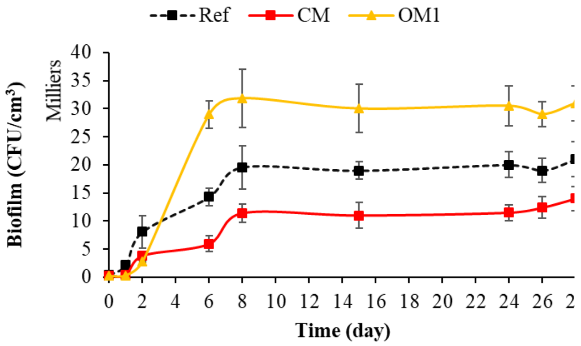

Figure 1. Quantification of bacterial colonization of mortar specimens. Each quantification was performed in triplicate using the culture-based method, and the error bars present the standard deviation from the values obtained. Ref = control mortar; CM = cured mortar; OM1 = oiled mortar prepared with CEM I.

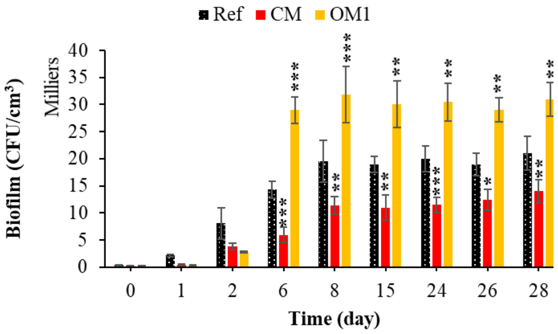

Figure 2. Statistical comparison (GraphPad Prism 5) of the biocolonization of mortar surfaces treated and untreated with curing compound or green formwork oil. Each experiment was performed in triplicate, and the error bars present the standard deviation of the values obtained. The experiments highlighted with asterisks differed significantly from the control (Bonferroni; **: $p < 0.01$, ***: $p < 0.001$) at the indicated time.

Figure 1 shows that the bacterial colonization of mortar surfaces started with a latency phase, followed by a phase of cell growth and accumulation on the surfaces and ending with a plateau phase. These colonization kinetics were also observed in most of the studies quantifying bacterial-biofilm formation on cementitious-material surfaces or on other surface types [27,28].

Figure 2 shows that the curing compound significantly inhibits the bacterial colonization of mortar submerged in seawater. Cells accumulated and grew faster and more extensively on an untreated surface. The compositions of curing compound used in this study indicate the presence of alkyl (C14-C18) bis (2-hydroxyethyl) amine, 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (FDS Sikacem cure). These elements or their derivatives have been cited as an anti-biofilm molecules that inhibited the formation and the accumulation of bacterial biofilm [35,36]. Moreover, the treatment of the material by an hydrophobic surface coating is cited as one of the anti-biofilm strategies used to inhibit bacterial and other organisms adhesion on the surface [37–39]. To verify the effect of the curing film on the hydrophobicity of the mortar surface, the contact angle with a drop of water was measured for the treated and untreated surface using a drop-shape scanner. From their preparation until the start of the immersion test, the mortars treated with the curing compound presented a contact angle greater than 110° (40° in the case of untreated mortars), indicating a hydrophobic surface [40]. After immersion and under the action of seawater, the contact angle gradually decreased with time and became equal to that of the control mortar at 28 days of immersion (data not shown). Therefore, we propose that the curing compound inhibited the biological colonization of mortar surfaces in seawater because of its chemical composition (anti-biofilm) and its effect on surface hydrophobicity.

However, Figure 2 shows that the formwork oil significantly promotes the bacterial colonization of mortar submerged in seawater. The composition of the green formwork oil used in this study is not given. Detailed information is given neither in the technical product information nor in the bibliography. Therefore, knowing that green formwork oil is biodegradable, we propose that this oil applied on the surface of the mortar specimens was used as a carbon source by marine bacteria, according to the study and the results of [41,42]. Dusane *et al*. showed by a laboratory test that the biofilm formation is affected by the carbon sources. Lactic acid, erythritol, glycerol, glucose and edible oils increase this process [43]. Therefore, we propose that the surface treatment with this type of formwork oil increased the biological colonization of cementitious materials in the marine environment because it is used as a nutrients source by marine microorganisms.

3.2. Effect of the type of cement and the surface roughness on the biocolonization of mortar specimens.

In order to compare and classify the intrinsic parameters that influence the biocolonization of cementitious materials in the marine environment, we also tested in this study under the same conditions the effect of the type of cement (the use of CEM III) and the surface roughness on the biofilm formation at the surface of mortar specimens.

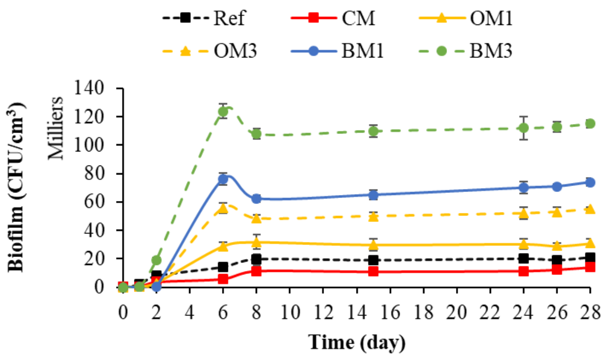

Figure 3. Quantification of bacterial-biofilm formation on mortar specimens. Each quantification was performed in triplicate using the culture-based method, and the error bars present the standard deviation from the values obtained. Ref = control mortar; CM = cured mortar; OM1 = oiled mortar prepared with CEM I; OM3 = oiled mortar prepared with CEM III; BM1 = biomimetic mortar prepared with CEM I; BM3 = biomimetic mortar prepared with CEM III.

Figure 3 shows that the bacterial colonization of mortar surfaces in the case of OM3, BM1 and BM3 has the same kinetic observed in the case of Ref, CM and OM1 (cf. Figure 1). However, the use of CEM III (BM1 vs. BM3 or OM1 vs. OM3) significantly increases the biofilm gormation. These results were in keeping with the literature, in which a similar effect of cement type on the biological colonization of cementitious materials has been reported [28,44,45]. In addition, a rough mortar surface significantly increased bacterial colonization in comparison with a smooth surface (Ref). This influence of surface roughness was identified in several studies concerning the biological colonization of cementitious materials [27,44].

Therefore, based on the rate of biological colonization (Figure 3), the mortar types can be classified from less to more bioreceptive in the following order: CM< Ref < OM1 < OM3 < BM1 < BM3. Then, the intrinsic parameters that promotes the biocolonization of cementitious materials in the marine environment can be classified from more to less effectively in the following order: surface roughness > the use of CEM III (type of cement) > surface treatment with green formwork oil.

However, the main cause of concrete structure deterioration in the marine environment is the chemical attack caused by seawater ions such as chloride and magnesium sulfate attack [13]. According to the literature, the biological colonization of immerged concrete structure can have a protective effect (bioprotection) against chemical attack in seawater, leading to improved structure durability [19,20]; the marine organisms adhered to the concrete surface form a physical barrier that reduces surface permeability. The decrease in surface permeability leads to less-efficient diffusion of aggressive ions (Cl^-, Mg2+ and OH^-), which can increase the durability of a concrete structure in the marine environment. Furthmore, Lv *et al*. demonstrate that the presence of the *Crassostrea gigas* coating (biological coating) on the surface of marine concrete can reduce the water absorption of concrete, and enhance the resistance of concrete to chloride penetration and carbonation [46]. Then, improving the biological colonization of concrete structure in the marine environment can have a positive effect both for the environment (biodiversity restoration) and for the concrete structure (bioprotection).

4. Conclusions

Eco-design of marine structures is a major focus for many researchers and construction companies working in marine environment, to enhance durability and also since few years to minimize and mitigate human impacts toward “no net loss” on biodiversity policies **[26]**. This study tests, compares and classify the intrinsic parameters that influence the biocolonization of cementitious materials in the marine environment. Regarding the parameters tested in the present work, we can summarize that: (i) work practices such as the use of a curing agent and/or formwork oil have an impact on biocolonization; the surface treatment with green formwork oil enhance the biocolonization whereas the application of curing agent has the opposite effect. (ii) The use of rough surface or slag cement CEM III increases the bioreceptivity of cementitious materials. (iii) Among the parameters examined, surface roughness proved to be the factor that promotes biocolonization most effectively.

These results could be taken up in future recommendations to enable engineers to eco-design more eco-friendly marine infrastructure and develop green-engineering projects.

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